European Journal of Plant Pathology **106:** 423–431, 2000.

Ultrastructural observations and DNA degradation analysis of pepper leaves undergoing a hypersensitive reaction to *Xanthomonas campestris* pv. *vesicatoria*

Annalisa Polverari[1], Roberto Buonaurio[1], Samantha Guiderdone[1], Mario Pezzotti[2] and Mario Marte[1,*]
[1]*Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy;* [2]*Dipartimento Scientifico e Tecnologico, Università degli Studi, Strada Le Grazie, 37134 Verona, Italy;* [*]*Author for correspondence (Phone: +39755856465; Fax: +39755856482; E-mail: mmarte@unipg.it)*

Accepted 7 February 2000

Key words: apoptosis, bacteria, chromatin condensation, DNA degradation analysis, plant, programmed cell death

Abstract

Ultrastructural details of the hypersensitive reaction induced by infiltration with avirulent race 2 *Xanthomonas campestris* pv. *vesicatoria* in pepper 'Early Calwonder-10R' leaves (incompatible interaction) are reported. Affected cells displayed plasmalemma undulations and disruption, lysis of the chloroplast membrane, degeneration of other organelles, general cytoplasm disorganisation and, often, protoplast shrinkage. The nuclei contained large masses of electron-dense material, apparently formed by chromatin aggregation. In many cases a single chromatin-like layer was deposited on the inner side of the nuclear envelope leaving a finely granular matrix in the centre of the nucleus; the nucleolus usually disappeared. The nuclear envelope was sometimes ruptured and the internal matrix leaked into the cytoplasm. The content of many affected cells eventually coagulated and became very electron-dense. The walls often collapsed. All these alterations were especially visible in spongy mesophyll cells at sites where bacteria occurred in the intercellular spaces. Although some of the nuclear and cytoplasmic alterations recall certain aspects of apoptotic cell death, molecular determinations did not reveal any DNA degradation in hypersensitively reacting tissues. The first cell alterations in leaves infected with the virulent bacterial race 1 (compatible interaction) were observed only 27 h after inoculation, when the cytoplasm of some cells showed limited internal disorganisation and plasmolysis at sites where bacterial colonies developed.

Introduction

Electron microscopy studies have been carried out into the hypersensitive reaction (HR) of plants to bacterial pathogens in various host–bacterial interactions, including xanthomonad infection in different plant species (Al-Mousawi et al., 1982; Brown et al., 1993). In particular, host cell alterations at the sites of bacterial multiplication, attachment of bacteria to host cell walls and pathogen encapsulation associated with the expression of HR have been investigated in cotton–*Xanthomonas campestris* pv. *malvacearum* (Smith) Dye (Al-Mousawi et al., 1982) and pepper–*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (Brown et al., 1993). Other studies of host cell responses to different *hrp* mutants of *X. campestris* pv. *vesicatoria* or to saprophytic strains of the same bacterium, have provided further information on papilla deposition and localisation of H_2O_2 production (Brown et al., 1995; 1998).

In recent years, hypersensitive cell death, including that triggered by bacterial pathogens, has been regarded as a form of programmed cell death (PCD), i.e. a process resulting from the execution of a genetic programme which in HR possibly depends upon host–pathogen recognition (Dixon et al., 1994; Dangl et al., 1996). That HR is a kind of PCD is further confirmed by studies on lesion mimic mutants of

Arabidopsis thaliana and other species which develop hypersensitive-like cell death in the absence of avirulent pathogens (Dietrich et al., 1994; Greenberg et al., 1994; Dangl et al., 1996; Kosslak et al., 1997). The most common form of PCD, extensively investigated in animals, is apoptosis. One of the earliest morphological cell alterations in apoptosis is the condensation of chromatin and its deposition on the inner side of the nuclear envelope. The nucleus then undergoes fragmentation and fragments are incorporated into protoplast portions called apoptotic bodies (Kerr and Harmon, 1991). Apoptosis often includes degradation of DNA into large fragments (Oberhammer et al., 1993a), which in most cases is followed by breakdown into multiples of 180–200 bp due to an internucleosomal cleavage of [ds]DNA (laddering) (Wyllie, 1980; Schwartzman and Cidlowsky, 1993).

This paper presents an ultrastructural description of the whole process of host cell death, including the early changes, the collapse phase and the final degeneration, recorded in the HR induced in pepper leaves by an avirulent race of *Xanthomonas campestris* pv. *vesicatoria*. Particular attention is given to nuclear alterations which resemble certain aspects of apoptosis. Furthermore, it is shown that such morphological features are not associated with any DNA degradation.

Materials and methods

Plants, bacteria and inoculation procedure

Pepper (*Capsicum annuum* L.) plants of the cultivar Early Calwonder-10R (ECW-10R), which carries the *Bs 1* resistance gene to *Xanthomonas campestris* pv. *vesicatoria*, were grown in sterilised compost-enriched soil in a greenhouse at 22–28 °C, under natural light conditions. About 40 days after sowing, the plants were transplanted and transferred to a growth chamber at 28 ± 2 °C, 60–70% RH, 65 μE m^{-2} s^{-1} illumination and 14 h light period.

The pepper race 1 (strain Xcv 82-8) and race 2 (strain Xcv E3) of *X. campestris* pv. *vesicatoria*, which are virulent and avirulent, respectively, to the pepper cv ECW-10R, were used as inoculants in all the experiments. Bacterial cultures, kindly provided by Prof. Dr. R.E. Stall, University of Gainesville, Florida, were maintained as suspensions in 15% glycerol at −80 °C. The inoculum was prepared by suspending in deionised water bacterial colonies grown for 48 h on nutrient agar at 27 ± 1 °C. The suspension was spectrophotometrically adjusted to 10^8 viable cells ml^{-1} ($OD_{600} = 0.3$). The 3rd–5th leaves of pepper plants at the 8th true leaf stage were completely infiltrated with the bacterial suspensions using a syringe with a 30 G needle. Plants whose corresponding leaves were infiltrated with water served as controls. Inoculated plants and controls were kept in the same growth chamber under the above environmental condition. Six separate experiments were carried out, three devoted entirely to electron microscopy studies and three to both analysis of DNA degradation and ultrastructural observations.

Electron microscopy

Inoculated leaves were collected, together with the corresponding control leaves, immediately after the first signs of collapse appeared in hypersensitively reacting plants (6–8 h after inoculation) in the EM only studies. Leaf samples were then collected from the same plants every 1–2 h thereafter until wilting (10–12 h post-inoculation). In addition, plants inoculated with the race 1 pathogen were sampled at 27, 34, 48, 96 h and 6 days post-inoculation to follow host ultrastructural changes during the compatible interaction. In the experiments where the DNA fragmentation was also investigated, leaf samples from all plants were collected every 2 h, starting from 2 h after inoculation up to leaf wilting in the incompatible interaction.

Large pieces cut from collected leaves were immediately vacuum infiltrated with 2.5% glutaraldehyde in 0.08 M phosphate buffer at pH 7.0. Although vacuum infiltration of the fixative may partially displace bacterial cells from their development sites in leaf tissues (Brown et al., 1993), this procedure was adopted as preliminary experiments had shown that, generally, it better preserved host cell ultrastructure, which was of paramount importance in this investigation. After 2 h fixation at room temperature, smaller pieces (0.5–0.8 × 0.8–1.0 mm) were cut from the fixed material and post-fixed in 1% OsO_4 in the same buffer for 2 h at room temperature. Pieces were then dehydrated in ethanol and embedded in a mixture of Epon and Araldite resins. Ultrathin sections from embedded materials were stained with uranyl acetate and lead citrate and observed under a transmission electron microscope (Philips EM 400).

DNA fragmentation analysis

Leaves from the same plants used for the microscopy studies (collected every 2 h after inoculation) were

flash frozen in liquid nitrogen and stored at −80 °C. The whole leaf blade was ground into a fine powder in liquid nitrogen in a cold mortar and the powder was collected in an Eppendorf tube. The DNA was extracted according to two different protocols (Ryerson and Heath, 1996; Young et al., 1997) and run on a 2% agarose gel. The same frozen powder was also used in pulse field gel electrophoresis, performed according to Mittler et al. (1997).

DNA in conventional electrophoresis gels was depurinated in 0.125 M HCl for 15 min, denatured in a solution consisting of 0.5 N NaOH and 1.5 M NaCl for 45 min and then neutralised in 1.0 M Tris–HCl pH 7.4 plus 1.5 M NaCl for 45 min. DNA in pulse field agarose gels was exposed to UV light for 1 min, then denatured and neutralised as above. The separated DNA was transferred to a nylon membrane (Hybond N Plus, Amersham) by the capillary transfer method for 48 h and membranes were then baked in an oven at 80 °C for 45 min.

DNA from nuclei isolated from pepper plants was used to produce a ^{32}P-dCTP (deoxycytidine triphosphate) probe with a random-priming kit from Pharmacia-Biotech, according to the manufacturer's instructions. Purification of nuclei was performed as described in D'Ovidio et al. (1992). The hybridisation was carried out at 65 °C overnight. The membranes were washed in SSC at decreasing concentrations from 6× to 0.1× (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate), adding 1% SDS to the lowest concentrations. Finally, membranes were exposed to X-ray films at −80 °C for 2 days.

Results

Symptoms of bacterial infection

Pepper (ECW-10R) leaves infiltrated with the race 2 of *X. campestris* pv. *vesicatoria* (incompatible combination) manifested the first signs of HR as slight tissue collapse 6–8 h after inoculation. Large areas of affected leaves turned greenish brown and then gradually withered and coalesced until almost the entire infected leaf blade had wilted and shrivelled (12–15 h after inoculation). No symptoms appeared on plants of the same cultivar inoculated with the race 1 (compatible combination) at the time at which HR occurs in the incompatible interaction. There was, however, a slight decrease in turgor 24–27 h after inoculation, which gradually evolved to water soaking, chlorosis, epinasty and leaf abscission 7–8 days after inoculation. No symptoms were recorded in control pepper leaves infiltrated with distilled water.

Ultrastructural observations

No ultrastructural alterations were observed at 2, 4 and 6 h post-inoculation in leaves infected with the bacterial race 2. Subsequently, hypersensitive cell death developed which occurred mainly in the spongy mesophyll tissues and affected the palisade layer less frequently. Slight cell changes consisting of plasmalemma undulation, chloroplast disorganisation and cytoplasm vesiculation occurred in mesophyll cells, especially at sites where bacteria were seen in the intercellular spaces, in sections from leaves showing early signs of hypersensitive collapse (Figure 1A). Most host cells of these leaf tissues manifested a considerable disorganisation of the chloroplast lamellar system at a more advanced stage of HR (Figure 2A). Degeneration of other organelles was at this stage sometimes associated with cell death. Electron-dense vacuolar precipitates, possibly polyphenolic and sometimes observed in control leaves, increased in volume and frequency in cells undergoing HR. The precipitates usually manifested as aggregates of large spherical globules with an irregular or spiny profile (Figure 2A). Membranous bodies were often seen in the vacuoles of these cells.

In all the experiments, hypersensitive cell death was accompanied by a particular nuclear degeneration pattern. Large masses of an electron-dense material which strongly suggested chromatin condensation and aggregation were first observed in degenerating nuclei (compare Figure 1C, D and B). Later a single homogeneous layer of chromatin-like material deposited on the inner side of the nuclear envelope and a lightly electron-dense, finely granular matrix remained visible in the centre of the nucleus (Figures, 1F, G, I and 2B). The nucleoli were usually absent. The nuclear envelope persisted almost unchanged (especially Figure 1H) although it sometimes ruptured in a limited portion and the internal matrix tended to extrude into the cytoplasm (Figures 1G and 2B).

In sections from wilting and shrivelling leaves, the walls of most affected cells tended to collapse (Figure 2C) and the cell content was very electron-dense, coagulated and, sometimes, shrunken in the final stage of HR (Figure 2B and C). Nuclei that displayed the above signs of degeneration were clearly evident, even in cells with completely coagulated, strongly electron-dense content whereas other organelles were usually difficult to distinguish (Figure 2B). Cells at

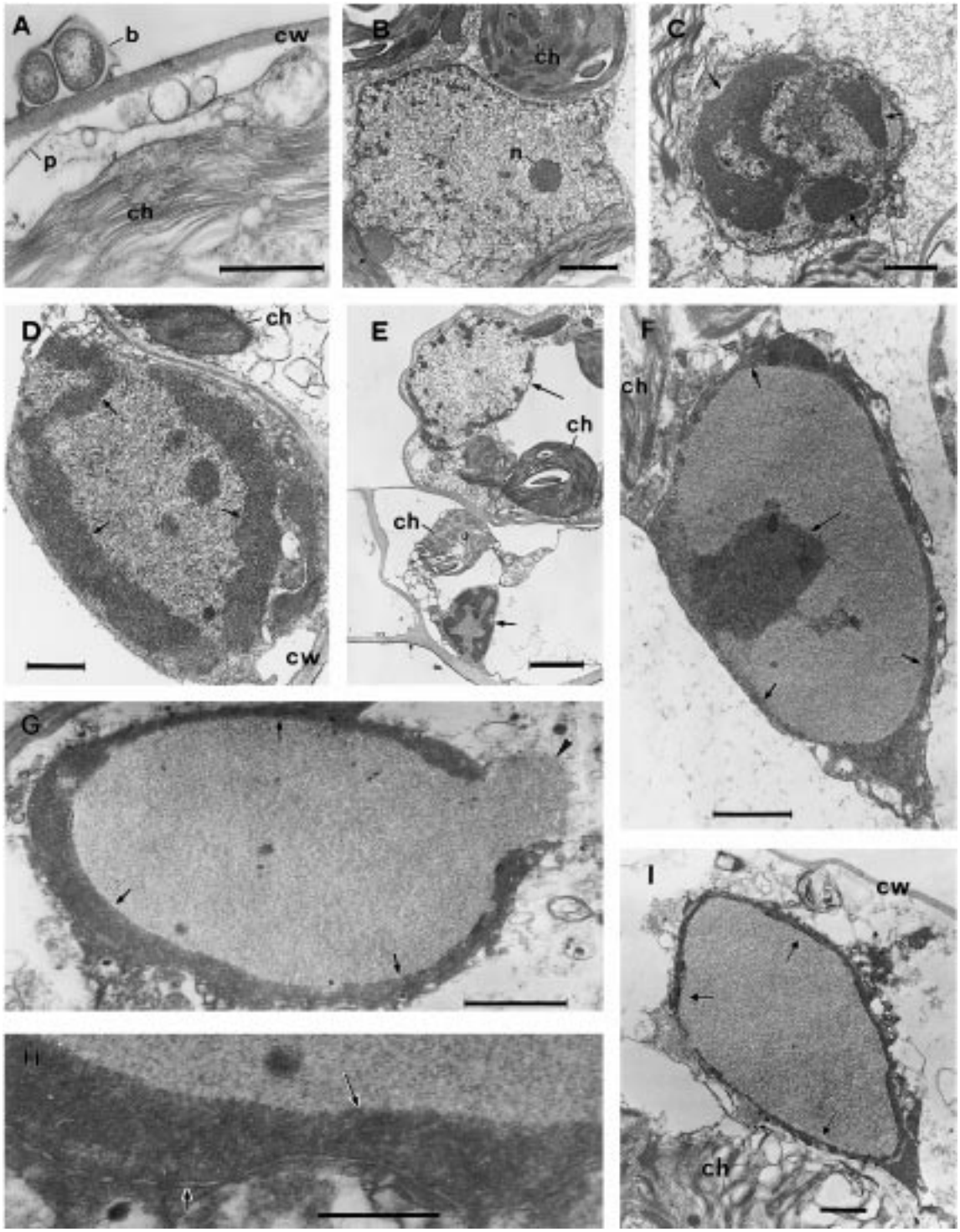

Figure 1. Ultrastructural details of hypersensitive reaction provoked by race 2 *Xanthomonas campestris* pv. *vesicatoria* in leaf cells of pepper (ECW–10R). (A) and (C) 6 h after inoculation or water infiltration; (B) and (D) to (I) 10 h after inoculation. (A) Plasmalemma alterations (detachment from the cell wall and formation of membranous vesicles) and incipient lamellar disorganisation of a chloroplast.

varying stages of alteration were observed at each collection time (Figure 1E).

There were no noteworthy structural or ultrastructural alterations in the leaves infiltrated with the race 1 pathogen (compatible interaction) at the time at which HR occurred in the incompatible interaction. Slightly disorganised cytoplasm and disrupted membranes were documented in scattered cells of the spongy parenchyma tissue of the leaves at the sites of bacterial multiplication 27 h post-inoculation (Figure 2D). Collapse and coagulation of mesophyll cells became evident during the subsequent evolution of the disease but nuclear changes similar to those associated with HR were never seen.

DNA degradation analysis

UV light visualisation of DNA separated on agarose gels by conventional and pulse field electrophoresis did not reveal the presence of distinct DNA fragments in the samples examined. In particular, no large size (50–300 Kb) or internucleosomal DNA fragments (ladder) were detected (Figure 3A). Southern blot hybridisation confirmed that the DNA was always intact, in both race 1- and race 2-infected leaves, even at the latest time point sampled (12 h) (Figure 3B), when EM showed a notable predominance of dead cells in the hypersensitively reacting (race 2-infected) leaves.

Discussion

The overall picture of host cell alterations observed in the present work shares general similarities with those previously reported in bacterial infections of plants (Brown and Mansfield, 1991; Brown et al., 1993; Goodman and Novacky, 1994; Bestwick et al., 1995; Fett and Jones, 1995) except for the absence of clearly defined papilla-like structures and the observed degeneration pattern of nuclei in hypersensitively reacting cells in our preparations. Paramural papilla-like formations are frequently detected on the inner side of host cell walls bordering intercellular spaces where phytopathogenic bacteria, including *X. campestris* pv. *vesicatoria*, are developing (Brown et al., 1993). The lack of papillae in the leaf material examined indicates that such structures may or may not be expressed in a single plant–pathogen interaction possibly depending on host cultivar–bacterial genotype combinations (Brown et al., 1995) and/or experimental conditions adopted.

The nuclear alterations and the protoplast shrinkage we observed in the hypersensitive cell death recall some aspects of the degeneration pattern characterising apoptosis. Morphological changes resembling apoptosis, such as protoplast shrinkage, plasma membrane blebbing and nuclear condensation have been observed in hypersensitively dying cells in other plant–pathogen interactions (Levine et al., 1996).

Apart from HR, examples of ultrastructural nuclear alterations suggestive of apoptotic cell death are also found in plant development, e.g. during degeneration of protophloem cells in wheat. An EM study showed that the nuclei of these cells exhibit typical chromatin condensation and undergo fragmentation (Eleftheriou, 1986).

The main differences between the morphological alterations observed in pepper–*X. campestris* pv. *vesicatoria* and typical apoptotic nuclear changes were the lack of nucleus fragmentation and the occasional occurrence of ruptures in the nuclear envelope (not described in typical apoptotic cell death). Regarding the other cytological features, long persistence of cytoplasmic organelle membranes is associated with apoptosis in animals, whereas early lysis of chloroplast membranes and general loss of cell compartmentalisation usually occurred in pepper leaf cells with apoptosis-like nuclear degeneration.

Note the two encapsulated bacterial cells (b) attached to the plant cell wall. Bar = 1μm. (B) Normal-looking nucleus and chloroplasts in a cell of a water-infiltrated control leaf 10 h after infiltration. Bar = 2μm. (C) and (D) Nuclei showing large masses of electron-dense material indicative of chromatin aggregation in hypersensitively reacting cells. Note the disorganised cytoplasm around the nucleus in (C). Bar = 1μm. (E) A normal-looking nucleus (long arrow) in an unaffected mesophyll cell and a degenerating nucleus (short arrow) in an epidermal cell undergoing HR in the same leaf section. Bar = 3μm. (F)–(I) Formation of a chromatin-like layer against the inner side of the nuclear envelope (arrows) in nuclei displaying apoptosis-like degeneration. A large, electron-dense mass (long arrow) in contact with the peripheral layer is still visible in (F). A lightly dense granular matrix can be seen in the central part of the nuclei. An arrowhead in (G) indicates the matrix leaking into the disorganised cytoplasm through a rupture of the nuclear envelope. Bar = 1μm. (H) Enlarged view of the nuclear envelope (short arrow) with the chromatin-like deposit inside (long arrow) shown in (G). Bar = 0.5μm; ch = chloroplast or chloroplast remnants; cw = plant cell wall; n = nucleolus; p = plasmalemma.

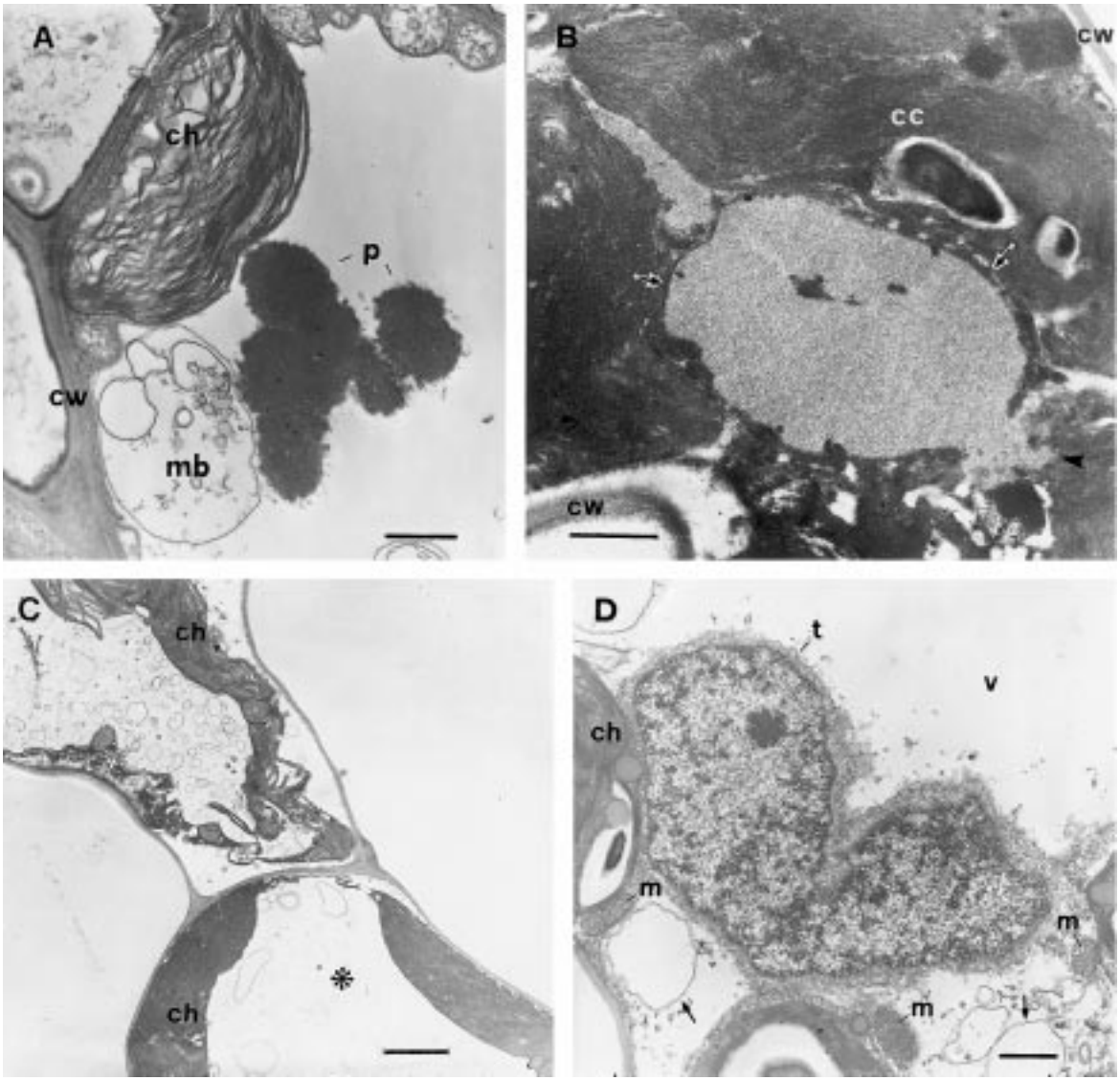

Figure 2. Ultrastructural changes in mesophyll cells of pepper ECW-10R leaves caused by *Xanthomonas campestris* pv. *vesicatoria* infection. (A) Degenerating cell in a race 2-infected leaf (incompatible interaction), 8 h after inoculation. Besides a marked chloroplast disorganisation, membranous bodies and aggregates of nearly spherical precipitates, with an irregular profile, are visible in the vacuole. Bar = 1μm. (B) and (C) Bacterial race 2-infection (incompatible interaction), 10 h after inoculation. What appears to be a degenerating nucleus (arrows) is still visible in the coagulated, very electron-dense content (cc) of a collapsing cell in (B). The nuclear matrix seems to extrude into the coagulated cytoplasm (arrowhead). Bar = 1μm. Cytoplasm disorganisation and protoplast shrinkage and slight wall collapse in a hypersensitively reacting mesophyll cell adjacent to a less severely damaged one (asterisk) in (C). Bar = 2μm. (D) Infection with race 1 (compatible interaction) 34 h after inoculation. Note the moderate cytoplasm disorganisation (arrows point to cytoplasmic vesicles) and partially disrupted tonoplast. The nucleus appears to be normal. Bar = 1μm; ch = chloroplasts or chloroplast remnants; cw = plant cell wall; m = mitochondrion; mb = membranous bodies; n = nucleolus; p = vacuolar precipitates; t = tonoplast; v = vacuole.

Another puzzling aspect observed in the present study is the absence of any DNA degradation, even in advanced stages of the hypersensitive cell death investigated. Formation of large DNA fragments has in fact been found in several plant cell death pathways (Koulaková et al., 1997; Mittler et al., 1997) including examples of HR mediated disease resistance (Levine et al., 1996; Mittler et al., 1997). Furthermore,

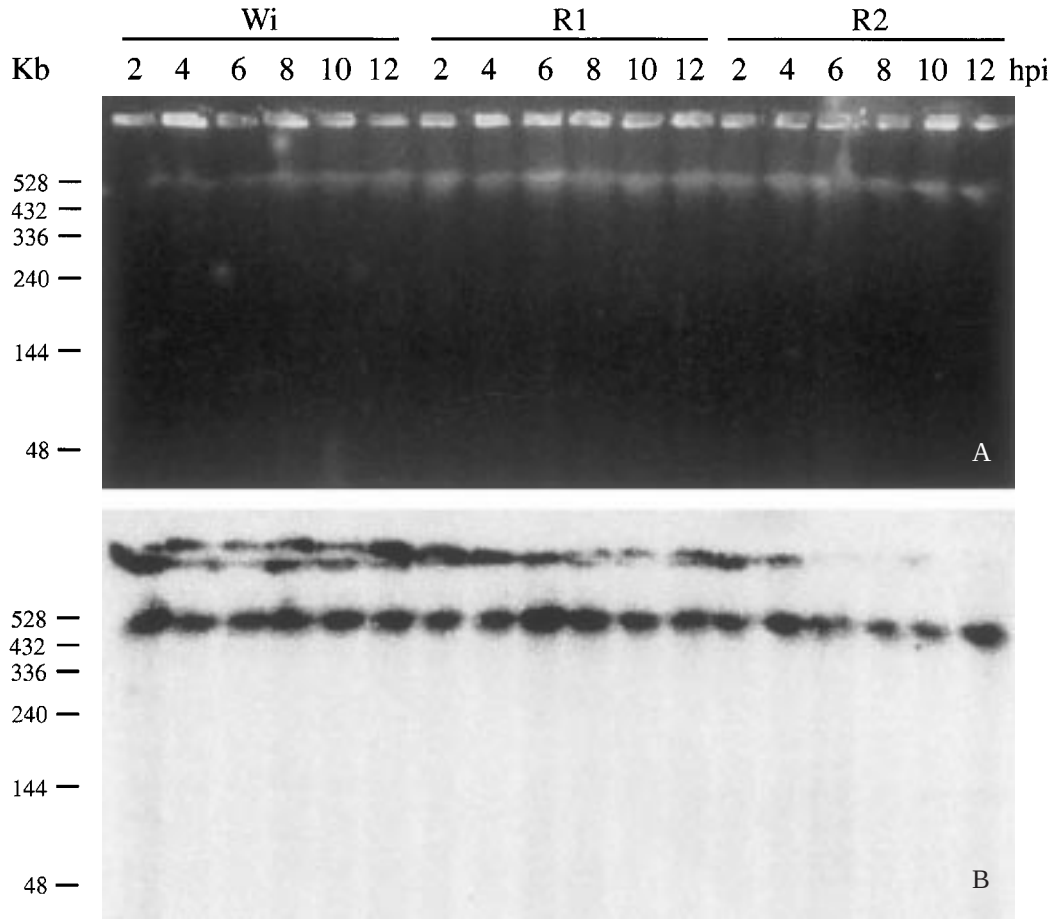

Figure 3. (A) Pulse field electrophoresis and (B) Southern blot analysis of pepper (ECW-10R) genomic DNA extracted from water infiltrated (control) leaves (Wi), and leaves inoculated with either race 1 (R1) or race 2 (R2) *Xanthomonas campestris* pv. *vesicatoria*, from 2 to 12 h post-inoculation. hpi = hours post-inoculation.

internucleosomal DNA cleavage has been reported in plant PCD (Young et al., 1997; Wang et al., 1996), among which pathogen-triggered hypersensitivity (Ryerson and Heath, 1996).

Also worthy of note is that in pepper HR to *X. campestris* pv. *vesicatoria* the lack of DNA degradation was found in the presence of morphological changes indicative of chromatin condensation. It has been suggested that apoptotic condensation of chromatin results from or is strictly associated with DNA degradation (Wyllie, 1980; Schwartzman and Cidlowsky, 1993). In plant PCD, including one case of HR, chromatin condensation and/or nuclear fragmentation appears indeed constantly associated with DNA breakdown (Levine et al., 1996; Wang et al., 1996; Kosslak et al., 1997; Koulaková, et al., 1997). However, this does not seem to be a general rule as experimental models are known in animals in which apoptotic morphology and DNA fragmentation can be dissociated, i.e. treatments inducing chromatin condensation do not necessarily lead to DNA fragmentation and *vice versa* (Oberhammer et al., 1993b; Sun et al., 1994).

In conclusion, in the incompatible interaction pepper–*X. campestris* pv. *vesicatoria* which we examined, only the pattern of chromatin condensation and protoplast shrinkage in hypersensitively reacting cells resembled apoptotic characteristics. Among the other examples of hypersensitive cell death sharing some, but not all features with animal apoptosis, is the TMV-induced HR in tobacco, where protoplast shrinkage and the formation of 50 kb DNA fragments were not associated with chromatin deposition on the nuclear

envelope (Mittler et al., 1997). Examples of HR are also known in which no apoptosis-like alterations are detected, the cell death being more similar to that which in animals is described as necrotic (e.g. Bestwick et al., 1995; Fett and Jones, 1995). It has been pointed out that the diversity of morphologies observed in cell death, including PCD, probably reflects different ways in which cells may die (Morel and Dangl, 1997). Apoptosis is the most widely conserved form of PCD in animals but, regarding the plant PCD, HR does not generally seem to fulfil all the morphological and molecular criteria that define an apoptotic process in a strict sense (Morel and Dangl, 1997). This was confirmed in the case of the incompatible interaction pepper–*X. campestris* pv. *vesicatoria* which we investigated.

Further information on the nature of plant PCD will be obtained from studies on important early markers of apoptosis recently established in animals, among which the activation of caspases (Ashkenazi and Dixit, 1998). The first available data on the involvement of these and other enzymes in plant cell death have already been published (e.g. Del Pozo and Lam, 1998).

Acknowledgements

This work was supported in part by a grant from MURST 40% (Progetto nazionale 'Ricerche epidemiologiche e fisiologiche sulle malattie crittogamiche delle piante') and in part by a CNR grant ('Indagini molecolari e citologiche sulla morte cellulare programmata nella resistenza per ipersensibilità delle piante a patogeni batterici'). We thank Prof. Rosalba Farnesi for her helpful suggestions and Judy Etherington for editorial assistance.

References

Al-Mousawi AH, Richardson PE, Essenberg M and Johnson WM (1982) Cotyledon and leaf ultrastructure of a bacterial blight immune cotton line inoculated with a low level of *Xanthomonas campestris* pv. *malvacearum*. Phytopathology 72: 1230–1234

Ashkenazi A and Dixit VM (1998) Death receptors: signaling and modulation. Science 281: 1305–1308

Bestwick CS, Bennet MH and Mansfield JW (1995) Hrp mutants of *Pseudomonas syringae* pv. *phaseolicola* induces cell wall alterations but not membrane damage leading to the hypersensitive reaction in lettuce. Plant Physiol 108: 503–516

Brown J and Mansfield J (1991) Interaction between *Pseudomonas* and *Phaseolus vulgaris*. In: Mendgen K and Lesemann DE (eds) Electron Microscopy of Plant Pathogens (pp 185–196) Springer-Verlag, Berlin

Brown I, Mansfield J, Irlam I, Conradsstrauch J and Bonas U (1993) Ultrastructure of interactions between *Xanthomonas campestris* pv. v*esicatoria* and pepper, including immunocytochemical localization of extracellular polysaccharides and the Avrbs3 protein. Mol Plant Microbe Inter 6: 376–386

Brown I, Mansfield J and Bonas U (1995) *hrp* genes in *Xanthomonas campestris* pv. *vesicatoria* determine ability to suppress papilla deposition in pepper mesophyll cells. Mol Plant Microbe Inter 8: 825–836

Brown J, Trethowan J, Kerry M, Mansfield J and Bolwell GP (1998) Localization of components of the oxidative cross-linking of glycoproteins and of callose synthesis in papillae formed during the interaction between non-pathogenic strains of *Xanthomonas campestris* and French bean mesophyll cells. Plant J 15: 333–343

D'Ovidio R, Tanzarella OA and Porceddu E (1992) Isolation of an α-type gliadin gene from *Triticum durum* Desf. and genetic polymorphism at the Gli-2 loci. Genet Breed 46: 41–48

Dangl JL, Dietrich RA and Richberg MH (1996) Death don't have no mercy: cell death programs in plant–microbe interactions. Plant Cell 8: 1793–1807

Del Pozo O and Lam E (1998) Caspases and programmed cell death in the hypersensitive response of plants to pathogens. Curr Biol 8: 1129–1132

Dietrich RA, Delaney TP, Uknes SJ, Ward ER, Ryals JA and Dangl JL (1994) *Arabidopsis* mutants simulating disease resistance response. Cell 77: 565–577

Dixon RA, Harrison MJ and Lamb CJ (1994) Early events in the activation of plant defence responses. Annu Rev Phytopathol 32: 479–501

Eleftheriou EP (1986) Ultrastructural studies on protophloem sieve elements in *Triticum aestivum* L. Nuclear degeneration. J Ultrastruct Mol Struct Res 98: 47–60

Fett WF and Jones SB (1995) Microscopy of the interaction of *hrp* mutants of *Pseudomonas syringae* pv. *phaseolicola* with a nonhost plant. Plant Sci 107: 27–39

Goodman RN and Novacky AJ (1994) The hypersensitive reaction in plants to pathogens: a resistance phenomenon. APS Press St. Paul, Minnesota.

Greenberg JT, Guo A, Klessig DF and Ausubel FM (1994) Programmed cell death in plants: a pathogen-triggered response activated coordinately with multiple defense functions. Cell 77: 551–563

Kerr JFR and Harmon BV (1991) Definition and incidence of apoptosis: and historical perspective. In: Tomei LD and Cope FO (eds) Apoptosis: The Molecular Basis of Cell Death (pp 5–31) Cold Spring Harbour Laboratory Press, New York

Kosslak RM, Chamberlin MA, Palmer RG and Bowen BA (1997) Programmed cell death in the root cortex of soybean *root necrosis* mutants. Plant J 11: 729–745

Koulaková B, Kovarík A, Fajkus J and Široký J (1997) Chromatin fragmentation associated with apoptotic changes in tobacco cells exposed to cold stress. FEBS Lett 414: 289–292

Levine A, Pennell RI, Alvarez ME, Palmer R and Lamb C (1996) Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Curr Biol 6: 427–437

Mittler R, Simon L and Lam E (1997) Pathogen-induced programmed cell death in tobacco. J Cell Sci 110: 1333–1344

Morel J-B and Dangl LD (1997) The hypersensitive response and the induction of cell death in plants. Cell Death Differ 4: 671–683

Oberhammer F, Wilson JW, Dive C, Morris ID, Hickman JA, Wakeling AE, Walker PR and Sikorska M (1993a) Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 50 kb fragments prior to or in the absence of internucleosomal fragmentation. EMBO J 12: 3679–3684

Oberhammer F, Fritsch G, Schmied M, Pavelka M, Printz D, Purchio T, Lassmann H and Shulte-Hermann R (1993b) Condensation of the chromatin at the membrane of an apoptotic nucleus is not associated with the activation of an endonuclease. J Cell Sci 104: 317–326

Ryerson DE and Heath MC (1996) Cleavage of nuclear DNA into oligonucleosomal fragments during cell death induced by fungal infection or by abiotic treatments. Plant Cell 8: 393–402

Schwartzman RA and Cidlowski JA (1993) Apoptosis: the biochemistry and molecular biology of programmed cell death. Endocr Rev 14: 133–151

Sun DY, Jiang S, Zheng LM, Ojcius DM and Young JD (1994) Separate metabolic pathways leading to DNA fragmentation and apoptotic chromatin condensation. J Exp Med 179: 559–568

Wang H, Li J, Bostock RM and Gilchrist DG (1996) Apoptosis: a functional paradigm for programmed cell death induced by a host-selective phytotoxin and invoked during development. Plant Cell 8: 375–391

Wyllie AH (1980) Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. Nature 284: 555–556

Young TE, Gallie DR and De Mason DA (1997) Ethylene-mediated programmed cell death during maize endosperm development of wild-type and shrunken 2 genotypes. Plant Physiol 115: 737–751